REMARKS/ARGUMENTS

After entry of this amendment, claims 13-16, 20-24, 27-32, 36-44, and 48-52 are pending in the present application. Each of the issues raised in the Office Action as they relate to the pending claims are addressed below in the order they are presented.

Claim Objections

The objection to claims 53, 57, 61, and 65 for allegedly being a substantial duplicate of claims 1, 13, 29, and 41 is rendered moot by cancellation of claims 53-68.

Rejections under 35 U.S.C. § 112, second paragraph

Pending claims 14-16, 30-32, and 42-44 were rejected for being indefinite because it is allegedly unclear whether the recited sequences are the native form of the DNA polymerase or the mutated form. Each of these claims have been amended to clarify that the ***native*** form of the enzyme comprises the recited sequence. Support for the claim amendments is found, for example, at page 4, line 23 to page 5, line 24. There, each of SEQ ID NOs: 2-7 are clearly identified as "the critical motif in the native form of the DNA polymerase." Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 102

Claims 13-14, 16, 29-30, 32, 41-42 and 44 stand rejected for allegedly being anticipated by Bergquist *et al.* (WO 95/14770). According to the Office Action, this reference discloses a wild type *Thermus filiformis* DNA polymerase having reverse transcriptase activity in the presence of magnesium. As also noted by the Examiner, this enzyme has the motif disclosed in SEQ ID NO: 4. As clarified above, the pending claims are directed to ***mutant*** DNA polymerases, which in their ***native*** form comprise the recited sequences. For example, claims 16 and 32 are directed to ***mutant*** enzymes derived from enzymes comprising SEQ ID NO: 4. Thus, since the Bergquist *et al.* publication fails to disclose mutant DNA polymerases as claimed here, the reference cannot anticipate the pending claims.

In addition, a careful reading of the reference shows that it is doubtful that the authors discovered the reverse transcriptase activity allegedly disclosed there. As explained

below, some of the primers allegedly used in the reaction do not hybridize to the mRNA molecule used in the amplification reaction (RNA transcribed from the *E. coli* α-lactalbumin gene, *see* page 11).

The Examiner points to page 12 of Bergquist for teaching a reverse transcription reaction. According to Bergquist, either primer T7 or primer P3 was used as the forward primer. The T7 primer corresponds to an *E. coli* phage T7 promoter sequence found in a large number of T7 *in vitro* transcription plasmids. For reverse primer P1, the 3'-most 20 nucleotides are complementary to the 20 3'-most nucleotides of the α-lactalbumin cDNA sequence. Primer T7 however, is not complementary to α-lactalbumin mRNA. Primers P1 and T7 could not be used to reverse transcribe and amplify an *in vitro* mRNA transcript from a plasmid comprising α-lactalbumin RNA, since the T7 promoter sequence is not within the *in vitro* mRNA transcript. Thus, no amplification using primer T7 could be accomplished. Therefore, the results in Fig. 7 of D2 cannot be believed to demonstrate efficient Mg^{2+}-activated reverse transcriptase activity by a *T. filiformis* DNA polymerase, because that is simply impossible.

The same applies to the experiments of Figure 8. The cellular α-lactalbumin mRNA sequence and the topoisomerase IIa sequence in total cellular RNA do not contain a nucleotide sequence corresponding to the T7 primer sequence. Therefore, they cannot demonstrate magnesium-activated reverse transcriptase-PCR activity from a total cellular RNA sample.

The rejection of claims 13, 20, 24-29, 36, 40-41,48 and 52 over Gelfand *et al.* (US Patent No. 5,374,553) is also respectfully traversed. This patent is cited for teaching a *Thermotoga maritima* DNA polymerase comprising a motif as shown in SEQ ID NO: 1. The Examiner notes that the residue in position 4 of the motif of this enzyme is not E, A, G, or P. As noted above, the claims are clearly directed to ***mutated*** enzymes, that have the properties recited. Since the enzyme described in the Gelfand patent is the ***native*** enzyme, it does not disclose or suggest the claimed enzymes.

The rejection of claims 13, 20, 24-29, 36, 40-41,48 and 52 over Gelfand *et al.* (WO 92/06202) is also respectfully traversed. This patent is cited for teaching a *Thermosipho africanus* DNA polymerase comprising a motif as shown in SEQ ID NO: 1. The Examiner notes

that the residue in position 4 of the motif of this enzyme is not E, A, G, or P. As noted above, the claims are clearly directed to ***mutated*** enzymes, that have the properties recited. Since the enzyme described in the Gelfand application is the ***native*** enzyme, it does not disclose or suggest the claimed enzymes.

The rejection of claims 13, 20, 24-29, 36, 40-41,48 and 52 over Swaminathan *et al.* (US Patent No.5,830,714) is also respectfully traversed. This patent is cited for teaching a *Bacillus stearothermophilus* DNA polymerase comprising a motif as shown in SEQ ID NO: 1. The Examiner notes that the residue in position 4 of the motif of this enzyme is not E, A, G, or P. As noted above, the claims are clearly directed to ***mutated*** enzymes, that have the properties recited. Since the enzyme described in the Swaminathan patent is the ***native*** enzyme, it does not disclose or suggest the claimed enzymes.

The rejection of claims 1-4, 8-10, 12, 53, and 61 for allegedly being anticipated by Gelfand *et al.* (EP 0902035 A2 or US Patent 6,346,379) is rendered moot by cancellation of these claims.

In light of the above, applicants respectfully submit that none of the references cited above disclose or suggest the claimed invention and that the rejections under 35 U.S.C. § 102 should be withdrawn.

Rejections under 35 U.S.C. § 103(a)

The rejection of claims 11, 13-16, 20-32, 36-44, 48-68 over the Gelfand references (EP 0902035 A2 or US Patent 6,346,379) in view of Kawasaki is respectfully traversed.

As explained previously, to establish a *prima facie* case of obviousness, the Examiner must meet three basic criteria. First, the Examiner must show that there is some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, the Examiner must show a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of

success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) and MPEP § 2142. To support the rejection, the examiner must "present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references." *Ex parte Clapp*, 227 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985) and MPEP § 2142.

In the present rejection, the Examiner acknowledges that neither of the primary references discloses the use of magnesium, primers and DNA polymerases in reverse transcription reactions of the invention. The teachings of Kawasaki are relied on to provide this missing teaching. As explained in the previous response, those of skill recognized that certain thermoactive DNA polymerases had sufficient reverse transcriptase activity in the presence of manganese, but ***not*** magnesium, which was too inefficient to be practical for RT-PCR techniques.

As noted above, a proper rejection under 35 U.S.C. § 103(a) must provide reasoning or evidence to show that one of skill would have a reasonable expectation of success in carrying out the claimed invention. In the present case, the Examiner must provide some reasoning or evidence to show why one of skill would have a reasonable expectation that DNA polymerases of the invention would be sufficiently active in the presence of magnesium. The Examiner points to nothing in the cited references that even remotely suggests that the enzymes taught by Gelfand would be useful in the buffer described in Kawasaki. In the absence of such a showing, the rejection is clearly improper and should be withdrawn.

In the Office Action, the Examiner states that the above argument is not persuasive because the claims are not drawn to any particular concentration of magnesium. The Examiner goes on to assert that one of skill would have been motivated to include magnesium in the reaction buffer because of the general recognition that magnesium is desirable in RT/PCR reactions. Applicants respectfully submit that the Examiner is correct about the desirability of magnesium in ***PCR*** reactions (*i.e.*, without a reverse transcription step). As explained in the specification, however, there was nothing in the art that showed that a single enzyme could be used for an reverse transcription ***and*** PCR using a magnesium buffer.

In particular, the Examiner's attention is drawn to page 1, line 27 to page 2, line 2. There it is explained that the prior art taught that the commonly used thermostable DNA polymerase, Taq polymerase, is inefficient is reverse transcription reactions magnesium buffers. At page 2, line 1 to page 3, line 2 it is explained that PCR amplification starting with an RNA template were thus carried out by first reverse-transcribing the target RNA using a mesophilic (*i.e.*, ***non-thermostable***) retroviral reverse transcriptase and then amplifying the resulting cDNA using a thermostable DNA polymerase.

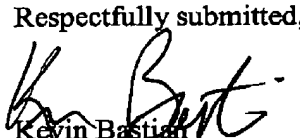
Later, a significant advance was achieved with the discovery that a thermostable DNA polymerase could be used to efficiently reverse transcribe an RNA template by carrying out the reaction in a manganese buffer, rather than a magnesium buffer. Efficient manganese-activated reverse transcription using a thermostable DNA polymerase is well described in the art (*see* U.S. Patent Nos. 5,310,652; 5,322,770; 5,407,800; 5,641,864; 5,561,058; and 5,693,517). As both the synthesis of cDNA from an RNA template and the synthesis of DNA from a DNA template can be carried out in a manganese buffer, the use of a manganese buffer enables single-enzyme, coupled reverse transcription/amplification reactions.

The mutant thermostable DNA polymerases of the invention, in contrast, allow the use of single-enzyme, coupled reverse transcription/amplification reactions in ***magnesium*** as opposed to ***manganese***. The Examiner has provided no evidence that enzymes capable of carrying out this reaction were either described or suggested in the art. Indeed, the Examiner has pointed to no reference in which an enzyme capable of carrying out such a reaction is even suggested. In the absence of a showing applicants respectfully submit the present rejection is improper and should be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at 415-576-0200.

Respectfully submitted,



Kevin Bastian
Reg. No. 34,774

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, Eighth Floor
San Francisco, California 94111-3834
Tel: 415-576-0200
Fax: 415-576-0300
Attachments
KLB:klb
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